Trilogy of COVID-19: Infection, Vaccination, and Immunosuppression

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Abstract
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative respiratory pathogen responsible for coronavirus disease 2019 (COVID-19). In 2020, the power of open science was visible to all, as novel vaccinology led to rapid establishment of vaccine clinical trials, and subsequent authorization of SARS-CoV-2 at an unprecedented pace. This evoked rapid deployment of SARS-CoV-2 vaccines and booster doses to keep with the ever-changing landscape of SARS-CoV-2. In this review, we provide an overview of vaccine efficacy studies, which have been well characterized in healthy individuals. Nevertheless, vaccine efficacy within the immunosuppressed is less well characterized, as these individuals were omitted from initial efficacy studies. Consequently, vaccine-induced responses in this group are relatively unknown. Currently, limited evidence investigating vaccine efficacy within the immunosuppressed is available. Here, we provide an overview of SARS-CoV-2 infection and associated pathogenesis. Furthermore, we undertake a critical analysis of observed vaccine responses from clinical studies, conducted in healthy and immunosuppressed populations. Whilst vaccine deployment has curbed mortality, there are significant challenges that lie ahead. This includes correlating vaccine responses with protective immunity and ensuring that global vaccine equity is met.

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has caused substantial loss of life and widespread disruption to lives and socio-economic infrastructures. Whilst authorization has been provided for several vaccines, we enter an arms race to immunize mankind whilst adapting our practices to the ever-changing mutant strains. Vaccination, alongside non-pharmaceutical intervention, represents the optimal way to control this pandemic. We are fortunate that we

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Fig. 1. Mechanism of SARS-CoV-2 infection. SARS-CoV-2, a betacoronavirus belonging to the *Orthocoronavirinae* family, uses both endosomal and non-endosomal pathways to infect host cells. Within endosomal entry, SARS-CoV-2 spike protein attaches to host ACE2 receptor. Following binding, TMPRSS2 cleaves spike subunits, leading to both fusion of viral and cell membranes. Following endocytosis, SARS-CoV-2 disseminates to release nucleocapsid and viral RNA into the cytoplasm required for translation and replication. Subsequent translation of viral proteins is then assembled within the endoplasmic reticulum to form new virions. Virions are packaged within Golgi vesicles, which are transported to cell surface and released from the cell via exocytosis. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2. Images created with BioRender.com.
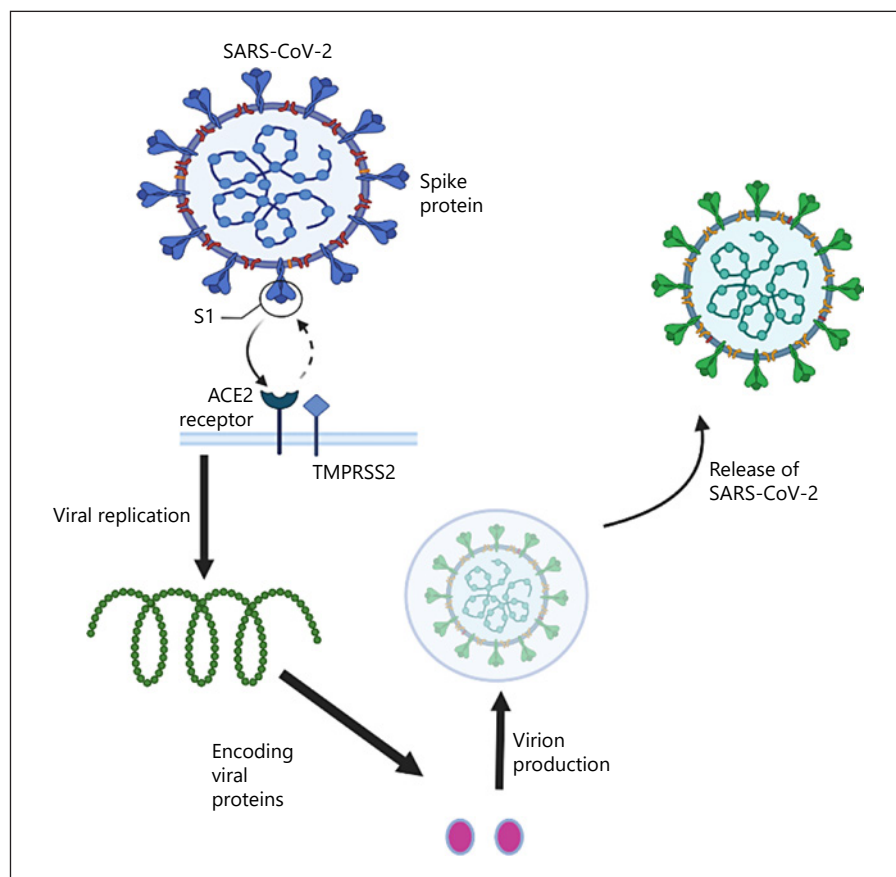
have an arsenal of potential vaccines in development. Of the 330 vaccine candidates that have been proposed so far (December 2021), 136 are in clinical development, 95 have entered phase III trials, and 25 have received some form of authorization for human use.

Whilst SARS-CoV-2 vaccine efficacies have been characterized in healthy individuals, immunogenicity in the clinically vulnerable remains ambiguous. Moreover, there is an urgent requirement for large-scale studies to investigate the impact of immunomodulatory therapies on vaccine responses. In this review, we cover the basic biology and immunopathogenesis of SARS-CoV-2. We also detail the current literature available on the vaccine-induced responses within the immunosuppressed population.

Immune Responses to SARS-CoV-2 Infection

Viral Entry

The initial step in infection is virus binding to host cell via its target receptor (Fig. 1). As with SARS-CoV, the target receptor for SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2) [1]. Expression of ACE2 is ubiquitous among epithelial cells in oral mucosa, liver, kidney, intestine, and heart [2, 3]. Work conducted on SARS-CoV highlighted that viral infection downregulates ACE2 expression within the alveolar epithelial cells [4]. Additionally, as ACE2 regulates the renin-angiotensin system (RAS) [5], viral infection could render RAS dysfunction, leading to hypotension, electrolyte imbalances, enhanced inflammation, and vascular permeability within the respiratory tract. Therefore, ACE2 downregulation has been positively correlated with acute lung injury and immunopathology in COVID-19 [6, 7].

Nevertheless, epidemiological characteristics of COVID-19 have highlighted that severe COVID-19 pathology could be attributed in individuals with existing comorbidities [8]. These include hypertension, diabetes, chronic obstructive lung disease, and coronary heart disease [8–10]. Pinto et al. [11] conducted a meta-analysis, which highlighted that genes encoding an ACE2 receptor within the lung parenchymal tissue are upregulated in in-

dividuals with such comorbidities. Using Pearson-correlation analysis, the group identified 544 genes, which were positively correlated with ACE2 expression. Among these, *ADAM10* and *TLR3* were identified, which plays vital roles in regulating the cleavage of ACE2 in human airway epithelia and viral innate immune responses, respectively. The authors stipulated a higher ACE2 receptor expression within the lung epithelia, in individuals with comorbidities, facilitated enhanced SARS-CoV-2 entry into respiratory tract during infection. Subsequently, higher viral loads translate into more severe disease phenotype, as supported with other studies [12, 13]. Whilst such causal link may be plausible, the findings of this study did not include COVID-19 infection data. Therefore, expression levels of ACE2 receptor may be a crucial regulator in disease progression. However, further work elucidating the cellular intricacies involving ACE2 expression and severe COVID-19 disease is required.

Through metagenomic analysis using next-generation sequencing, it was shown that SARS-CoV-2 shares 79.6% of sequence genomic identity with SARS-CoV [14]. Additionally, both comprise the spike (S) protein on the virion surface, giving its characteristic crown appearance. S-proteins are homotrimeric class I fusion glycoproteins, which are divided into two subunits: S1 and S2. S1 subunit is surface exposed, which contains the receptor-binding domain (RBD), which engages with ACE2, thus dictating both virus cell tropism and pathogenicity [15], whereas the S2 subunit consists of the fusion peptide (FP) region comprising two heptad repeat regions: HR1 and HR2 [16]. These heptad regions are a key structural feature of fusion proteins. HR1 is located downstream and within the vicinity of the FP, whereas HR2 occurs adjacent to the transmembrane region. RBD binding to ACE2 elicits SARS-CoV-2 virion endocytosis, consequently exposing it to endosomal proteases [17]. Subsequently, endosomal-mediated cleavage of S1 exposes the FP, which inserts itself into the host-cell membrane. This evokes S2 to fold in on itself, which brings together the HR1 and HR2 regions. The folded HR1 interacts with HR2 to induce a six-helix bundle, which brings together the viral membrane and host-cell membrane in close vicinity, which enables membrane fusion and dissemination of viral constituents into the host cytoplasm. Moreover, S-proteins consist of furin-cleavage sites, which are proteolytically targeted by cellular proteases, such as TMPRSS22, which further facilitates host-cell entry [18]. TMPRSS2 is widely expressed within the human respiratory tract and, thus, contributes to SARS-CoV-2 spread and pathology.

Innate Immune Response

SARS-CoV-2 and other respiratory coronaviruses, such as SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), are single-stranded RNA viruses. Following host-cell entry, viruses are recognized by pattern-recognition receptors (PRRs), such as toll-like receptors (TLR) 3, 7, 8, and 9, and viral sensors such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5) [16] (Fig. 2). Using TLR3 as an example, TLR3 binding to SARS-CoV-2 RNA triggers transcription of the Nod-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) gene. Alongside NLRP3 transcription and other cellular responses to viral infection, such as reactive oxidative species, calcium influx, and release of danger-associated patterns evoke the formation and activation of the NLRP3 inflammasome [18]. NLRP3 inflammasome causes capsase-1-dependent cleavage and induces proinflammatory mediators such as interleukin-1β (IL-1β) and IL-18, consequently triggering gasdermin D-mediated cell death. Within context of SARS-CoV-2 infection, monocytes and lung tissues consist of NLRP3 and apoptosis-associated speck-like protein along with a caspase-activating and recruitment domain. These are indicative of NLRP3 inflammasomes, which are produced in COVID-19 patients [19].

Interestingly, the level of NLRP3 activation positively correlates with elevated lactate dehydrogenase release [19] and has been found in high levels in coronavirus disease 2019 (COVID-19) with increased severity [20]. A characteristic feature of SARS-CoV, SARS-CoV-2, and MERS-CoV is the delayed induction of type I IFN release from infected cells [21, 22]. This delayed release has shown to contribute to the immunopathology, as it enables the virus to replicate and elicit further tissue damage, triggering a more exuberant immune response, as the immune system is overwhelmed with elevated viral load and apoptotic cells. Ensuing immune pathology occurs as inflammatory cells seep into the respiratory tract releasing further proinflammatory cytokines, further escalating an already inflammatory environment. Such hyperinflammation induces the so-called cytokine storm, consequently evoking acute respiratory distress syndrome. Such imbalanced responses along with delayed type I IFN induction are most likely factors contributing to the overall severity of acute COVID-19.

Inhaled SARS-CoV-2 viral particles are primarily deposited within the nasal mucosa, where infection and viral replication occur within the sinonasal airway epithelium. The upper airways are characterized by mucosal-
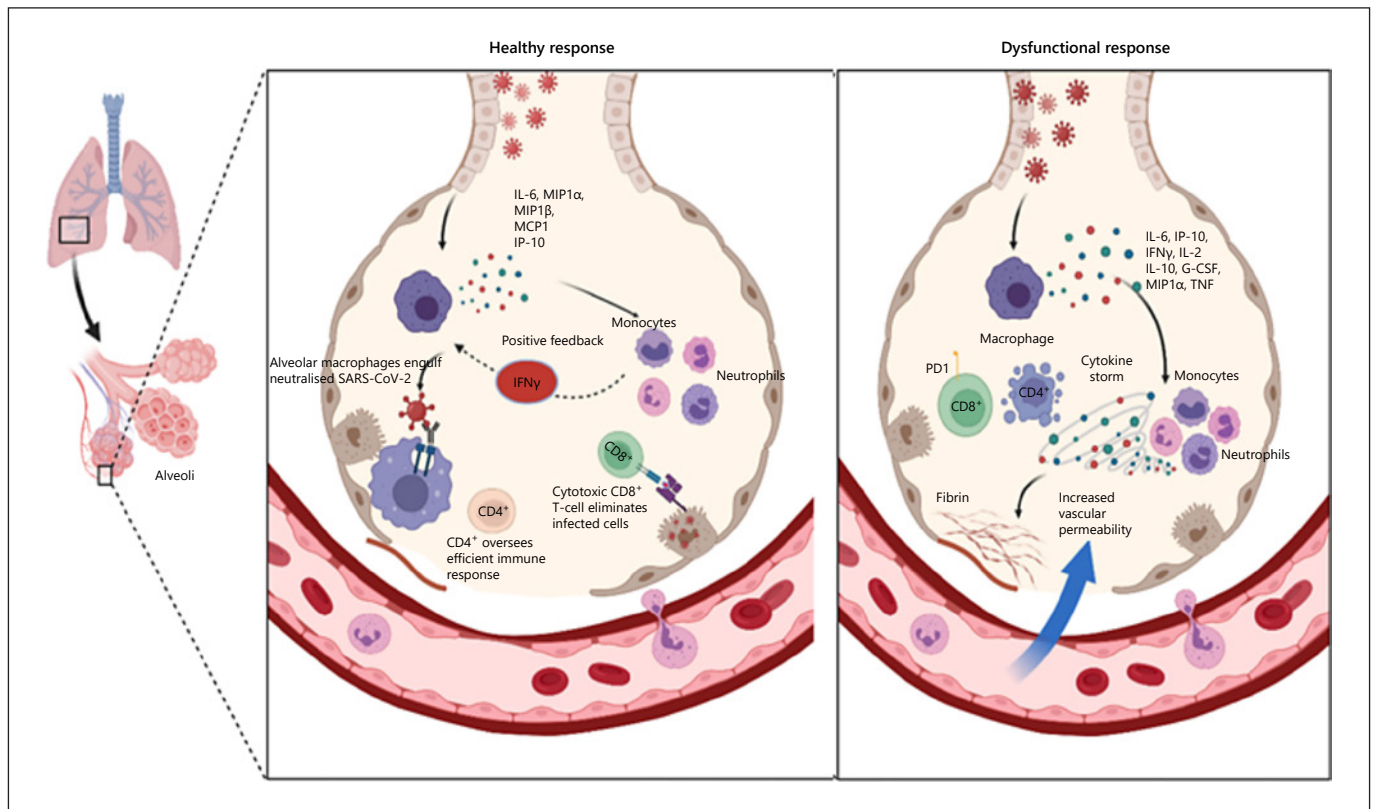
Fig. 2. SARS-CoV-2 pathogenesis. SARS-CoV-2 infects alveolar epithelial cells, which express ACE2 and TMPRSS2. During this active replication cycle, alveolar epithelial cells, macrophages, and endothelial cells detect viral PAMPs (viral RNA) and damage-associated molecular patterns, such as ATP and nucleic acids, through PRRs such as TLR7. This leads to TLR downstream signalling evoking proinflammatory cytokine production such as IL-6, IP-10, MIP1α, MIP1β, and MCP1. This proinflammatory environment recruits peripheral immune cells such as macrophages, monocytes, and T cells to site of infection, which heightens the inflammatory state within the site of infection. Inflammation is further heightened by the release of IFNY, which elicits a positive proinflammatory feedback loop. In a healthy response (left), this inflammatory state recruits SARS-CoV-2-specific T cells to site of infection. CD8+ cytotoxic cells (CTLs) recognize SARS-CoV-2 peptides, presented via class I MHC molecules, on type II pneumocytes. This causes CTLs to release IFNY, perforin, and granzyme B which eliminates SARS-CoV-2-infected pneumocytes, thus preventing viral spread. CD4+ T cells, especially Tfh, provides T cells help to B cells which stimulates neutralizing antibody production to SARS-CoV-2, consequently blocking viral infection. Furthermore, alveolar macrophages phagocytose both neutralized SARS-CoV-2 and apoptotic cells. Cumulatively, these cellular mechanisms lead to viral clearance with minimal upper respiratory damage, leading to resolution. Dysfunctional responses (right), which is marked by lymphopenia and CD4+ apoptosis, is characterized by a polyclonal antibody response, which is ineffective against neutralizing SARS-CoV-2. Furthermore, increased amounts of exhausted T cells express PD-1, which contributes to decreased proliferation and cytofunctionality of CD8+ T cells. Such impaired cytotoxicity results in chronic viral shedding, which exacerbates macrophage activation, leading to massive cytokine production, referred to as cytokine storm. Such deleterious uncontrolled inflammation leads to ARDS. Overall, chronic viral replication along with hyperinflammation leads to systemic vascular damage and disseminated intravascular damage, causing multiorgan failure and fatality. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme 2; TMPRSS2, transmembrane protease serine 2; PAMPs, pathogen-associated molecular patterns; RNA, ribonucleic acid; ATP, adenosine triphosphate; TLR, Toll-like receptor; IL-6, interleukin-6; IP-10, interferon Y-induced protein 10; MIP1α, macrophage inflammatory protein 1-alpha; MIP1β, macrophage inflammatory protein 1-beta; MCP1, monocyte chemotactic protein-1; PD-1, programmed cell death protein-1; ARDS, acute respiratory distress syndrome. Images created with BioRender.com.

associated lymphoid tissue of the Waldeyer's ring, which includes the nasopharynx-associated lymphoid tissue [23]. The sinonasal airway plays a central role in the induction of innate immune responses following pathogen invasion. Within this context, nasopharynx-associated lymphoid tissue represents the first lymphoepithelial barrier against airborne pathogens, such as SARS-CoV-2 [24]. As mentioned, SARS-CoV-2 is a cytopathic virus

capable of inducing cellular injury to infected epithelial cells. Such cellular insult is a result of SARS-CoV-2 inducing a highly inflammatory form of cell death called pyroptosis [25]. This process leads to wide-scale secretion of IL-6, IFN-γ, MCP1, and IP-10 into the circulation. Furthermore, damage-associated molecular patterns, induced by pyroptosis, are recognized by PRRs expressed widely on respiratory epithelial cells of the upper airway. Subsequently, downstream signalling via PRRs, such as endosomal TLR3 and TLR7, or cytoplasmic RIG-I, causes the activation of transcription factors, such as interferon-regulatory factor 3, interferon-regulatory factor 7, and NF-Kβ. This elicits transcription of type I and III IFN and cytokines such as IL-1β [26]. Consequently, this induces the antiviral proinflammatory innate immune response.

Evidence demonstrates that innate immune responses to SARS-CoV-2 are dampened in the upper airways, which can be attributed to disease severity. Single-cell RNA sequencing of cells derived from nasal brushings of COVID-19 patients demonstrated undetectable type I or type III transcripts, whilst antiviral genes *MX1* and *IFITM3* were expressed [27]. Furthermore, multiple cytokine secretions were assessed following SARS-CoV-2 infection of nasal epithelial cells. With exception of CXCL10, cytokine secretion was dampened [28]. Such innate immune response was substantially reduced from that observed in influenza A infection [28]. The reason behind the dampened interferon response to SARS-CoV-2 infection is yet to be elucidated. It has been postulated that the host microbiome and historical pathogen exposures could lead to such dampened innate immune response. The nasopharynx is the entry point for various inhaled viruses, allergens, and antigens, along with supporting an extensive microbiome. Such chronic exposure to microbial PAMPs could lead to dampened innate immune responses. Finally, the lower temperature within the sinonasal and epithelial surface may contribute to the dampened immune responses, as V'kovski et al. [29] demonstrated that SARS-CoV-2 grew at higher titres at 33°C than at 37°C, in airway epithelial cells, which was correlated with reduced interferon response.

Adaptive Immune Responses

Convalescent COVID-19 individuals demonstrate SARS-CoV-2-specific antibodies along with CD4+ and CD8+ T-cell responses. These humoral and cellular adaptive branches each have protective roles in controlling viral infections. The contribution of each branch in protective immunity is dependent on the type of viral trigger. Some virus infections demonstrate dominance to a specific branch, whilst others elicit a degree of synergy. For these reasons, each branch in terms of productivity and pathogenicity will be discussed in context of SARS-CoV-2.

The association of SARS-CoV-2-specific T cells with protective responses was first shown by the work conducted by Tan et al. [30], where SARS-CoV-2 viral RNA within the respiratory tract was quantified in parallel with antibodies and SARS-CoV-2-specific T cells for both structural (spike, nucleocapsid, and membrane) and non-structural (ORF7/8, NSP7) proteins. Findings demonstrated that early induction of IFNγ-secreting SARS-CoV-2-specific T cells was present only in mild COVID-19 disease patients and was associated with robust viral clearance. Nevertheless, there were some limitations in this work; firstly, the number of samples (*n* = 12) used to draw conclusions were too small. Moreover, the use of an ELISpot test to enumerate only IFNγ-secreting cells prevents the possibility of detecting SARS-CoV-2 T cells which are polyfunctional and produce multiple cytokines such as TNF and IL-17. Furthermore, the use of this single functional assay may prevent detection of functionally exhausted T cells, which may provide greater insights into mechanism of COVID-19 severity and viral clearance. Importantly, it must be highlighted that several studies have reported the opposite, where antibody and T-cell kinetics were positively correlated with disease severity [31, 32]. For instance, polyfunctional and robust SARS-CoV-2-specific T cells have been observed in severe COVID-19 patients compared to those with milder cases [33, 34]. A plausible explanation for discrepancies in the magnitude of T-cell responses could be that severe COVID-19 patients have a prolonged exposure to heightened viral loads.

Recently, a study by Hagin et al. [35] investigating SARS-CoV-2 vaccine immunogenicity in 26 patients with inborn errors of immunity demonstrated detectable SARS-CoV-2-specific T-cell responses within 73% of this cohort (19/26). Cellular responses were evaluated using an ELISpot assay, which contained a pool of lyophilized peptides of spike or membrane glycoprotein. These peptide pools covered the immunodominant sequence domains of spike, whilst encompassing the complete sequence of membrane glycoprotein. Interestingly, those patients who failed to seroconvert (*n* = 5) due to agammaglobulinaemia and B-cell-depleting therapeutics (rituximab) demonstrated strong T-cell responses. This is indicative that absent antibody responses do not mean a lack of protection. The validity of these findings from a prospective outlook remains elusive as samples were col-

lected from patients during the early post-vaccine period (2 weeks following vaccination). Consequently, no prediction can be made to determine if these immunological responses would be long-lasting.

Studies have demonstrated that CD4+ T-cell responses are prominent than CD8+ [36, 37] and are associated with primary control of SARS-CoV-2 infection [38]. Antigenic-specific CD4+ T cells have a plethora of functions. They have the capacity to differentiate into a range of T-helper (Th1) and effector subsets. For instance, virus-specific CD4 T cells differentiate into Th1 and T-follicular helper cells (Tfh). TfH provides T cell help to B-cells in key processes such as affinity maturation and memory B-cell differentiation. These processes play a vital role in the production of neutralising antibodies and long-term humoral immunity. [39]. Nevertheless, work conducted by Meckiff et al. [40] demonstrated the heterogeneity of CD4+ T-cell subsets, which are reactive to SARS-CoV-2. In this study, pools of lyophilized peptides encompassing the immunodominant regions of spike glycoprotein and a complete sequence of membrane glycoprotein of SARS-CoV-2 (15-mer sequences with 11 amino acids overlap) were used. Using the transcriptomic analysis of >100,000 viral antigen-reactive CD4+ T cells, the authors demonstrated increased proportions of SARS-CoV-2-reactive cytotoxic Tfh in hospitalized COVID-19 patients. This cytotoxic Tfh phenotype was shown to be negatively correlated with neutralizing antibody responses. Such findings are supported by previous studies, which demonstrated that cytotoxic Tfh kills B cells and suppresses germinal centre responses [41]. However, no negative association between cytotoxic Tfh and antibody responses was observed in non-hospitalized patients. Consequently, the potential inhibitory role of cytotoxic Tfh in antibody responses cannot be generalized. Moreover, detection of cytotoxic Tfh in hospitalized patients could reflect prolonged viral exposure and IFN production in severe disease. Further work is required to investigate the immunomodulatory response of cytotoxic Tfh in humoral responses to SARS-CoV-2.

During acute COVID-19 infection, CD8+ T-cell responses develop rapidly [38], with a broad spectrum of antiviral functions. SARS-CoV-2-specific CD8+ T cells have shown a robust cytotoxic effector phenotype, with elevated expression of effector molecules, such as IFN, granzyme B, perforin, and CD107a [37, 38]. This was exemplified by Schulien et al. [42], where 66 SARS-CoV-2-derived epitope peptides were tested in 26 patients with prior mild SARS-CoV-2 infection. Using in silico predictive modelling, 5 of the best matched peptides, based on the most frequent HLA class I alleles, were identified. Synthetic peptides were created and loaded into HLA class I tetramers to deduce CD8+ T-cell responses. Findings demonstrated that 88.5% (23/26) of patients elicited a robust SARS-CoV-2-specific CD8+ T-cell response covering a median of four epitopes. Moreover, SARS-CoV-2 CD8+ T cells were still detectable in seronegative convalescent individuals through the longitudinal follow-up, supporting that the notion humoral immunity wanes faster compared to cellular responses. However, the assessment of immunodominance could not be derived from this study, as the epitopes do not cover the entire SARS-CoV-2 viral genome, which is usually resolved through the use of overlapping peptides [36]. Recent studies have demonstrated that SARS-CoV-2 CD8+ T cells are specific for various ranges of SARS-CoV-2 structural and nonstructural proteins [36, 43, 44]. Overall, the above findings provide a parsimonious working model that coordinated cellular and humoral responses are protective in SARS-CoV-2 infection, whereas uncoordinated responses fail to control the infection leading to impaired adaptive responses, which succumb to immunopathology observed in COVID-19.

Heterogeneity, Demographics, and SARS-CoV-2 Pathogenesis

Discussion of the contribution of socio-economic risk factors to COVID-19 severity is out of scope of this review and can be found elsewhere [45]. However, it is prudent to amalgamate the fundamental risk factors with immune pathology observed in SARS-CoV-2 infection. Heterogeneity is a key determinant in COVID-19 severity and immune responses to SARS-CoV-2. The human immune system is inherently heterogeneous and varies significantly on an individual level. Heterogeneity can be observed in SARS-CoV-2 infections and elicited immune responses, as there is a broad range of disparity in COVID-19 severity, and innate and adaptive immune responses to SARS-CoV-2 [46]. Consequently, it is vital to impart heterogeneity into the equation when drawing conclusions pertaining to SARS-CoV-2 immunity and disease.

Nevertheless, certain risk factors have been well characterized in severe COVID-19. Age is the largest risk factor, which is positively correlated for severe or fatal COVID-19. For instance, a 65-year-old individual has a 70× greater risk of death from COVID-19 when compared to a 20-year-old individual [47]. Moreover, the vascular

condition, during infection, between the young and the elderly is markedly different. This observation could be explained as ageing individuals have a lower repertoire of naïve T cells capable of polarizing responses towards a specific antigen, which has been well characterized [48, 49]. Moreover, in SARS-CoV-2, COVID-19 severity was inversely correlated with the repertoire of naïve T cells detected. Together, these data demonstrate the importance of T-cell response against severe COVID-19, whereby the older individual elicits a slower T-cell response, enabling SARS-CoV-2 to have a head-start, consequently, inflicting wide-scale immunopathology.

Males are at a somewhat greater susceptibility in developing severe COVID-19 than females, whilst some hypothesize the hormonal influence of oestrogen in females provides antiviral and anti-inflammatory protection against COVID-19 [50]; to date, no clear functional differences have been found [51]. Nevertheless, a large-scale study conducted by Bastard et al. [52] ($n = 2,877$) explored the clinical variability in COVID-19. Strikingly, they found that 95/761 COVID-19 severe patients had developed autoantibodies (auto-Abs) against type I IFNs and were male. In contrast, only 2.6% of female patients had generated type I IFNs auto-Abs. The authors concluded that auto-Abs against type I IFN impairs both innate and adaptive branches resulting in severe COVID-19 pneumonitis. Whilst this skew in males is striking, the ethnicities of majority of severe COVID-19 patients were undisclosed, with Europeans forming the majority within the disclosed ethnicities. Therefore, with past misattribution between physiological, genetic, and racial factors, it is prudent to set a high threshold when drawing conclusions between demographical factors to COVID-19 severity.

As already discussed within immunological response section of this review, infection and destruction of respiratory cells elicit a local immune response, which recruits further innate and adaptive immune cells to the site of infection. In most cases, this process resolves the infection; however, in certain circumstances, a dysfunctional immunological response occurs, resulting in severe respiratory, systemic pathology, and fatality. Cytopathic viruses, such as SARS-CoV, MERS-CoV, and SARS-CoV-2, cause death and injury of both infected cells and tissues as part of their replicative cycle. Both viral replication and subsequent proinflammatory cytokine release, such as IL-1β, are linked with SARS-CoV-2 pyroptosis [53] – an inflammatory form of cell death and critical trigger for ensuing inflammatory process [54]. As stated before, detection of SARS-CoV-2 PAMPs and damage-associated molecular patterns by PRRs present on alveolar epithelial

cells and macrophages triggers downstream signalling required for secretion of proinflammatory cytokines and chemokines such as IL-6, IFN, monocyte chemoattractant protein 1 (MCP1), and interferon gamma-induced protein (IP-10) [55, 56]. The cytokine signature correlates with a Th1 cell-polarized response, analogous to both SARS-CoV and MERS-CoV [57]. The local inflammatory milieu within the local microenvironment attracts monocytes, macrophages, and T-lymphocytes to site of infection [58, 59]. Interestingly, neutrophils are not recruited as predominantly compared to T-lymphocytes, which may explain the lymphopenia and elevated neutrophil-lymphocyte ratio seen in around 80% of COVID-19 patients [60].

In mild COVID-19 responses, the above processes clear the infection within the upper respiratory tract, with receding immune responses and resolution. However, in some individuals, a dysfunctional response is triggered, which is characterized by hyperinflammation and ensuing cytokine storm within the upper respiratory tract. Studies from acutely unwell COVID-19 patients have identified significantly higher levels of IL-2, IL-7, IL-10, granulocyte colony-stimulating factor, IP-10, MCP1, macrophage inflammatory protein 1-alpha, and TNF [61]. Moreover, IL-6 levels were significantly elevated in those that had fatal outcomes compared to survivors [9]. Such observation led to therapeutic intervention, with IL-6 inhibitors (tocilizumab) being licensed for the treatment of severely unwell COVID-19 patients. The RE-COVERY trial [62], which evaluated the effects of tocilizumab on hospitalized ICU COVID-19 patients ($n = 4,116$), reported a statistically significant improvement in survival outcomes in patients receiving tocilizumab, yet with a very modest reduction of case mortality when compared to those receiving usual care (31% vs. 35% compared with usual care, $p = 0.0028$). It begs the question, given that IL-6 levels are positively correlated with COVID-19 severity and mortality, why do IL-6 antagonists not significantly improve survival? Dorgham et al. [63] shed light into this miscorrelation, as it was shown that mortality within severe COVID-19 patients ($n = 28$) was not associated with a single cytokine, rather it was a combination of elevated TNF, IL-6, IL-8, and low type I-interferon response. COVID-19 patients with moderate disease, who still required hospitalization, but not mechanical ventilation, had both higher inflammatory and type I IFN responses. These findings could explain why treatment with glucocorticoids, such as dexamethasone, was successful across all hospitalized COVID-19 cohorts (i.e., those requiring and not requiring mechanical venti-

Table 1. Vaccine efficacy results from phase III trials

Vaccine manufacturer (name)	Vaccine type	Regime used	Number of trial participants	Efficacy	Eligibility	Endpoints	Data by disease severity
Pfizer-BioNTech (BNT162b2) [70]	mRNA	2 doses (21 days interval)	43,548	95%	>16 years (years) old	Symptomatic and RT-PCR positive	100% and 95.3% effective against CDC and FDA defined severe disease
Moderna (mRNA-1273) [71]	mRNA	2 doses (28 days interval)	30,420	94%	≥18 years old, if: 12–18 years enrolled to NCT04649151 6 months to 12 years, then NCT04796896	Symptomatic and RT-PCR positive	100% against severe disease
AstraZeneca-University of Oxford (AZD1222) [72]	Viral vector	2 doses, 6-week interval2 doses, 12-week interval	17,178	55% for 6-week 81%, 12 weeks Pooled: 67%	≥18 years old Not pregnant	Symptomatic and RT-PCR positive	100% against severe disease
Johnson & Johnson (Ad26. COV2-S) [73]	Viral vector	1 dose	44,325	66%	≥18 years old	Symptomatic and RT-PCR positive	85.4%
Gamaleya (Sputnik V) [74]	Viral vector	2 doses, 21 day interval	19,866	92%	≥18 years old	Symptomatic and RT-PCR positive	100%

RT-PCR, reverse transcriptase polymerase chain reaction; CDC, Centre for Disease Control; WHO, World Health Organization.

lation), as modulation and activity of several proinflammatory cytokines – rather than IL-6 only – could contribute to its efficacy. Nevertheless, the small sample size of this study ($n = 44$) should be taken into consideration. Moreover, clinical trials utilizing this personalized precision medicine approach are required to determine if personalized cytokine profiling may improve outcome.

Subtle phenotypic changes in innate immune cells are also observed in severe COVID-19 patients [64, 65]. Specifically, severe COVID-19 patients exhibit a higher inflammatory phenotype, as evident by greater proportions of monocyte-derived FCN1+ macrophage and CD14+ CD16+ inflammatory monocytes. Alterations within the T-lymphocyte compartment were also demonstrated by Biasi et al. [66], where severe COVID-19 patients ($n = 21$) exhibited increased markers of senescence and exhaustion (CD57+ PD1+), altered cell proliferation, and a skew towards Th17 phenotype. However, this study was limited by the low sample number of which patients were enrolled based on their clinical characteristics. This experimental design does not provide sufficient statistical power for extensive analysis. Moreover, this does not enable the authors to compare altered cellular phenotypes between severe and mild disease. Blood samples would have been collected from patients who had received therapeutic intervention. Therefore, it is challenging to draw conclusions of immune modifications, which could be due to natural infection or therapy induced.

The above functional and phenotypic alterations contribute to the hyperinflammatory state, which causes a state of cytokine storm. This has shown to cause systemic pathology such as sepsis and multiorgan failure, due to a combination of myocardial damage and circulatory failure [67]. This vicious hyperinflammatory cycle prolongs the state of vascular permeability within the alveoli, evoking further inflammatory cell infiltration. Cumulatively, excessive cytokines, proteases, and reactive oxygen species cause direct tissue damage, evoking diffuse parenchymal alveolar damage, alveolar cell desquamation, hyaline membrane accumulation, and pulmonary oedema [58, 59]. Pathophysiologically, this causes acute respiratory distress syndrome and COVID-19 pneumonitis, through inefficient gas exchange and subsequent hypoxia. Overall, crucial inroads into local and systemic immunopathogenesis have been established. However, amalgamation of these cellular processes with demographic factors is required to map out the deleterious inflammatory responses, which may in turn optimize COVID-19 therapy.

SARS-CoV-2 Vaccine Responses

Fifteen months from the inoculation of the first experimental COVID-19 vaccine dose in humans, there is now a global race to roll out vaccines and booster doses to achieve herd immunity and subsequently control the pandemic [68]. Despite EMA and Food and Drug Administration having authorized four and three vaccine candidates, respectively, the pandemic is far from over, as evident by the ever-emergent SARS-CoV-2 variants of concern. Here, we

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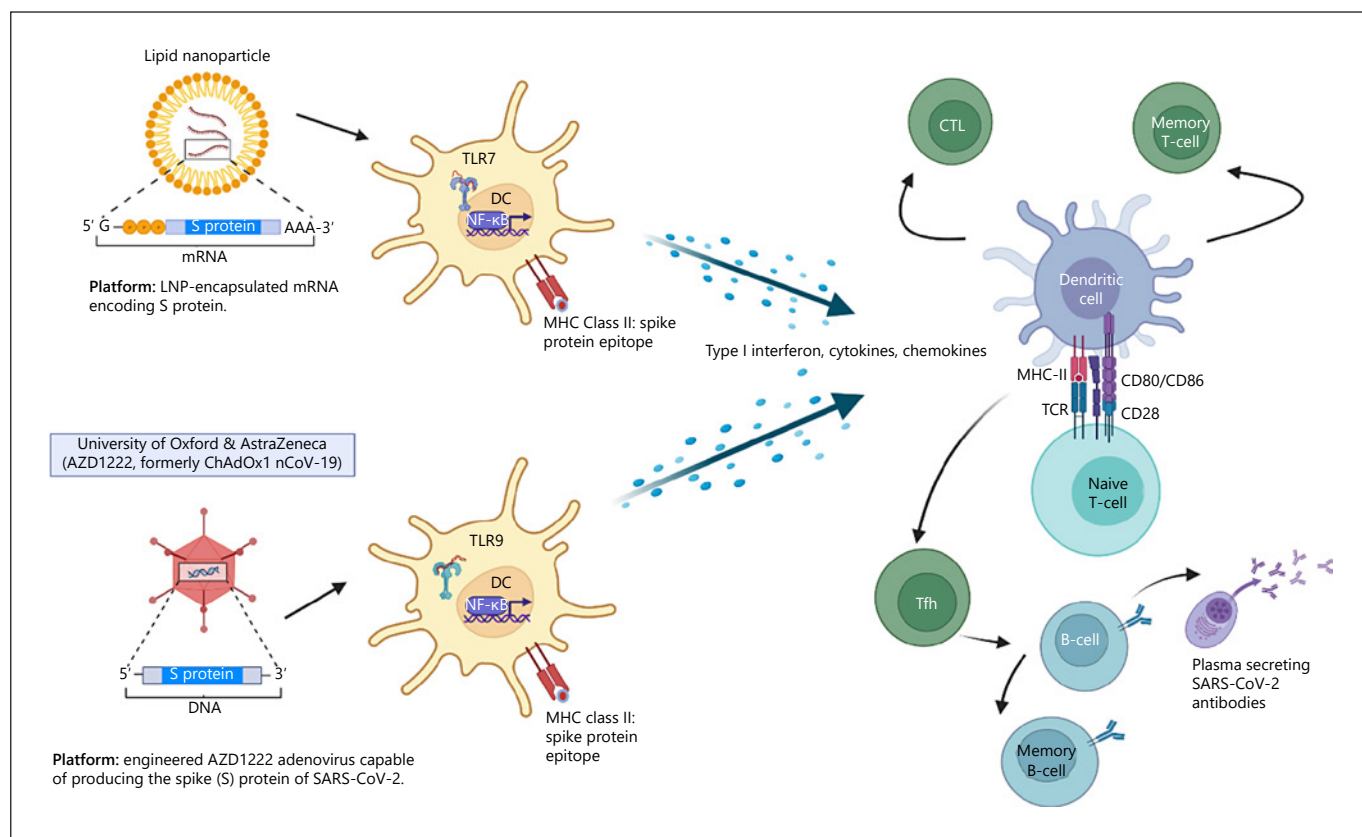
Fig. 3. Adaptive immune responses elicited by mRNA and adenoviral vector vaccines. Both vaccine platforms, mRNA and adenoviral vectors, encode the Spike protein. mRNA (encapsulated within lipid nanoparticles) and Adv enter DCs within the inoculation site or lymph nodes. This elicits translation of SARS-CoV-2 genetic material to produce spike protein. Furthermore, PRRs, such as TLR7 (mRNA) and TLR9 (Adv), detect the adjuvants in both vaccines, leading to proinflammatory cytokine production and DC activation. Consequently, activated DCs present the antigenic pep-tide along with costimulatory signalling molecules to naïve T cells, rendering SARS-CoV-2-specific T-cell activation. These activated T cells then differentiate into effector cytotoxic T cells and Tfh; the latter provides T cell help to activated B cells, which help drive germinal centre reactions and the production of high-affinity anti-spike antibodies. Following vaccination, peripheral blood comprises circulatory SARS-CoV-2-specific memory T and B cells, along with anti-spike antibodies, which provides protection against SARS-CoV-2. Images created with BioRender.com.

will briefly touch on the mechanisms of immunogenicity elicited by the vaccines, implications of their durability and protective immunity to healthy and diseased populations.

Current Vaccine Landscape

Innovative and open science along with industrial partnerships has led to authorization of two main vaccine types for emergency use. The first is represented by mRNA technology as formulated by Pfizer-BioNTech (BNT162b2) [69] and Moderna (mRNA-1273) [70]. Non-replicating recombinant adenoviral vector (Adv) represented the other, as illustrated by AstraZeneca-University of Oxford (ChAdOx1 nCoV-19) [71], Johnson & Johnson (Ad26. CoV2-S) [72], and Gamaleya (Sputnik V) [73]. The efficacies of these vaccines are summarized in Table 1. Comparisons between the efficacy of different vaccine platforms have been conducted [74, 75]. Data have suggested that mRNA platforms have a higher efficacy (94–95%) than AdV (67–92%) [74, 75]. Understanding the differences in vaccine efficacies between platforms is instrumental in optimizing and developing successful vaccines for both current and future pandemics. As illustrated in Table 1 (vaccine efficacy table), efficacies have ranged from 67% to 95%, but we postulate that such differences could be due to differences in trial design, assessment of endpoint measured, trial location, demographics of participants, and prevalent SARS-CoV-2 variants at time of study. Due to such complex variables, it is not feasible to conduct evaluative comparative analysis between vaccine platforms. It could be argued that developing ef-

fective vaccines within the remits of the ongoing pandemic is of more importance than comparative studies of these effective vaccines, as the latter could potentially delay vaccine authorizations and compound infection numbers and fatalities.

Mechanisms of Vaccine-Induced Immunity

An optimal vaccine design requires a pathogen-specific immunogen and adjuvant (Fig. 2). The latter should be effective enough to stimulate innate immune responses whilst providing secondary signals required for T-cell activation (Fig. 3). Moreover, adjuvants should be selected based on their capacity to offset undesired systemic inflammation from innate responses, which could evoke severe side effects. For mRNA vaccines, immunogen and adjuvants are represented by the mRNA, as it consists of the genetic material required to encode the spike protein (immunogen), whereas the immunostimulatory properties of RNA act as the adjuvant. As single-stranded (ss) and double-stranded RNA (ds) enters the cell, they are detected by various endosomal and cytosolic PRRs, such as TLR3, 7, MDA5, and RIG-I; the latter detects both ss-RNA and dsRNA [76]. This leads to downstream PRR signalling eliciting cellular activation, type I IFN production, and several proinflammatory mediators [76]. To offset undesired systemic inflammation, the purified mRNA is modified with nucleotides, which reduces binding to PRRs, consequently, preventing overstimulating this inflammatory response [76]. Moreover, the viral mRNA is encased within a lipid nanoparticle, which promotes cellular translation of mRNA into spike protein. Lipid nanoparticles act as carriers of the mRNA to lymph nodes. Here, dendritic cells (DCs) engulf lipid nanoparticles, which enable mRNA to be transfected into DCs via endocytosis [77]. The entrapped mRNA undergoes endosomal escape and is released into the cytosol. Using the host cellular machinery, such as ribosomes, the mRNA is translated into antigenic proteins and is degraded into antigenic peptides by proteasomes. These peptides are transported from the cytoplasm to endoplasmic reticulum by the transporter for antigen processing [78], which is a subunit of the MHC class I loading complex and is involved in MHC class I loading, in order to increase the efficiency of the process. Once peptide is bound successfully, MHC class I molecule is released from the loading complex and is loaded onto the surface of DCs. Activation of DCs is augmented by the inflammatory environment created by PRR signalling. Activated DCs present the processed spike peptide to naïve T cells, subsequently priming SARS-CoV-2-adaptive responses.

In Adv vaccines, the viral DNA is encapsulated within a non-replicating adenoviral vector, the adenoviral particles acting as the adjuvant. Following inoculation, adenoviral particles stimulate PRRs, especially TLR9, present on DCs and macrophages. TLR9 can specifically bind to dsDNA; thus, they are not engaged in mRNA vaccines. Nevertheless, like mRNA vaccines, PRR binding to dsDNA triggers TLR9 downstream signalling and elicits type I IFN production [79]. Within DCs and macrophages, S-protein is encoded by the vaccine-induced nucleic acids, which are expressed on the surface of activated DCs and macrophages. Antigen presentation of S-protein and subsequent costimulatory signalling leads to development SARS-CoV-2-specific adaptive immune responses. Both mRNA and Adv have capacity to drive intracellular spike protein production along with eliciting type I IFN production. This polarizes the CD8+ and CD4+ differentiation into effector and memory subsets. Such effector and memory subsets acquire the functional ability to secrete inflammatory and cytotoxic mediators, upon subsequent SARS-CoV-2 challenge. Moreover, CD4 Tfh is instrumental in inducing B-cell germinal centre reactions and differentiation of activated B cells into SARS-CoV-2 antibody-secreting plasma cells.

Vaccine Efficacy and Durability

As tabulated in Table 1, the efficacies for the listed vaccine types were obtained from clinical trials based on clinical trial-specific endpoints. Whilst these represent a critical measure when approving vaccines for human use, they do not reflect the efficacy within the real world. This is especially true as the trials have been conducted on mostly young and healthy adults. Pregnant and immuno-compromised individuals have been excluded from these studies. It is vital to understand the durability and longevity of these vaccine responses in all age and disease groups. The latter is particularly important in patients receiving immunosuppressive agents, such as rituximab or tacrolimus, where there are insufficient evidence-based data that these individuals are protected. This question is of higher importance given their clinical vulnerability to severe disease from SARS-CoV-2 infection [80].

As mentioned above, clinical trials ascertain vaccine efficacy; however, vaccine effectiveness, which can be defined as the decrease in SARS-CoV-2 infection risk within vaccinated individuals, should be addressed. In the United Kingdom, a study of vaccine effectiveness was carried out in elderly vaccinated patients (n = 156,930 aged 70 years or older) between December 2020 and February 2021 [81]. The effectiveness of by Pfizer-BioNTech and

AstraZeneca-University of Oxford vaccines was evaluated longitudinally in these participants. In patients aged 80 years or older, Pfizer-BioNTech vaccine effectiveness was 70% after first dose, which increased to 89% after the second dose. In the same study, it was shown that participants aged 70 years or over were 61% protected against symptomatic infection with Pfizer-BioNTech, with 60% protection from AstraZeneca-University of Oxford, 28–34 days after first vaccination. A limitation of this study is that controls were established differently; consequently, the odds ratio could be skewed. Moreover, the study used a test-negative design, where the control group is composed of individuals who present with SARS-CoV-2-like symptoms but test negative on RT-PCR. It is our belief that a screening method would be a more efficient design, where vaccination coverage in SARS-CoV-2-positive individuals is compared with vaccination coverage within the general population. This method may provide more robust information pertaining vaccine effectiveness, and such methods have been utilized in the SIREN study (SARS-CoV-2 immunity and reinfection) [82].

SARS-CoV-2 Vaccines and Adverse Effects

Both Pfizer-BioNTech and AstraZeneca-University of Oxford vaccines have demonstrated excellent safety and efficacy profiles in phase 3 trials. Within the community setting, safety and effectiveness of these vaccines were investigated in a prospective observational study [83] in the UK. Here, 627,383 vaccinated individuals (Pfizer-BioNTech: 47.3% and AstraZeneca-University of Oxford: 52.7%) self-reported systemic and local side effects within 8 days of vaccination. The most common self-reported systemic side effects were fatigue (Pfizer-BioNTech: 8.4% and AstraZeneca-University of Oxford: 21.1%) and headache (Pfizer-BioNTech: 7.8% and AstraZeneca-University of Oxford: 22.8%). Local adverse events were frequently reported as tenderness (Pfizer-BioNTech: 57.2% and AstraZeneca-University of Oxford: 49.3%) and local pain (Pfizer-BioNTech: 29.2% and AstraZeneca-University of Oxford: 19.1%).

Moreover, documentation of rare adverse events is vital for ongoing risk-benefit evaluations of current vaccination regimes and informing post-vaccination clinical practice. One of the reported adverse events includes the development of myocarditis following mRNA SARS-CoV-2 vaccinations. As demonstrated by the Vaccine Adverse Event Reporting System (VAERS), 1,226 reports of probable myocarditis/pericarditis have been reported following approximately 300 million SARS-CoV-2 mRNA vaccinations [84]. Males constituted 79% of these

reported cases, with a median age of 24 years. The time between onset of symptoms was a median of 3 days, where the highest rate occurred at day 2 following vaccination in patients aged 16–18 years of age. Several case reports of myocarditis following SARS-CoV-2 vaccination have been published [85–87], where patients present with chest pain, which is preceded with fever and myalgia. These case reports included young males, without the history of SARS-CoV-2 infection or comorbidities. Clinical findings included elevated cardiac enzymes, C-reactive protein, and ST elevations observed in electrocardiogram. Cardiac MRI was abnormal in all patients, with findings indicative of myocarditis, such as late gadolinium enhancement and myocardial oedema. Most of these patients resolved their symptoms with supportive care [85–87].

The exact immunopathogenesis of developing myocarditis, following mRNA SARS-CoV-2 vaccination, remains unclear. It is established that selected RNA molecules are highly immunogenic, which, if not modified appropriately, can cause mRNA destruction before it reaches target cells, consequently suppressing humoral responses. To overcome this, mRNA vaccine development includes nucleoside modifications of mRNA, which reduces the innate immunogenicity [88]. However, in certain genetically predisposed individuals [89], the immune responses to mRNA may not be adequately controlled, consequently evoking an aberrant innate and humoral immune response. The immune system may recognize the mRNA, within the vaccine, as an antigen. Subsequently, innate immune cells, expressing TLRs, can evoke the activation of proinflammatory pathways, which may drive the development of myocarditis, as part of a systemic reaction in susceptible individuals [88, 89].

Conversely, it can be argued that autoantibodies may induce the pathogenesis seen in SARS-CoV-2 vaccine-induced myocarditis. A case report [90] highlighted that autoantibody against self-antigens such as aquaporin 4, endothelial cell antigen, and proteolipid protein was observed in an affected patient. These autoantibodies were not observed in healthy individuals without myocarditis post-mRNA vaccination. It has been previously reported that cardiac autoantibodies develop in higher frequency in myocarditis patients, which may be pathogenic [91]. These autoantibodies could alter the functional effects on cardiac myocytes, which could explain one of the pathological mechanisms evoking myocarditis following vaccination. It should be highlighted that this patient's autoantibody levels peaked at day 2, along with clinical symptoms, but did not recede as expected following resolution.

Furthermore, autoantibodies in this patient could be transient due to myocardial inflammation. It was also evident that the patient had a surge in natural killer cells, along with elevated levels of IL-1 receptor antagonists, IL-5 and IL-16. However, proinflammatory cytokines, such as IL-6, TNF, and IFNγ, were not raised, findings that argue against a hyperimmune response. Subsequently, it is not clear whether the dysregulated cytokine profile, autoantibodies, and elevated natural killer cells are implicated in the pathogenesis or reactive response to myocardial inflammation. Such findings required validation in further studies with a larger cohort size. Another plausible mechanism for myocarditis is molecular mimicry between spike protein of SARS-CoV-2 and self-antigens [92]. Experimental models have shown that SARS-CoV-2 spike protein can cross-react with human peptide sequences, such as α-myosin [92]. Whilst SARS-CoV-2 vaccinations do not appear to evoke de novo immune-mediated adverse events, it cannot be ruled out that SARS-CoV-2 vaccinations could trigger dysregulated pathways in predisposed individuals. Such cellular aberrations could elicit a polyclonal B-cell expansion, immune complex formation, and inflammation.

Emerging reports of rare neurological complications have been linked with SARS-CoV-2 vaccinations, thus prompting clinical and public health concerns [93–99]. Additional case reports have also linked SARS-CoV-2 vaccinations to neurological adverse events, including Guillain-Barré syndrome (GBS) [95, 96]. However, case reports are often limited by small numbers along with selection and recording biases. Therefore, Patone et al. [100] performed a large population-based study in the UK, which involved more than 32 million people. They examined the neurological adverse events associated with Pfizer-BioNTech and AstraZeneca-University of Oxford vaccines. The group concluded that an increased risk of hospital admission (38 excess cases per 10 million exposed, within 1–28 days risk period) for GBS, Bell's palsy and myasthenic disorders was observed in those who received the AstraZeneca-University of Oxford vaccine. No observable risk of GBS was attributed to those who had received Pfizer-BioNTech vaccine. The authors further concluded that GBS and Bell's palsy co-occur in those who had received AstraZeneca-University of Oxford vaccine. Currently, it is unclear how AstraZeneca-University of Oxford vaccine is associated with the pathogenesis of GBS. Further work is required to examine whether antibodies against the adenovirus vector of AstraZeneca-University of Oxford vaccine can cross-react with components of the peripheral nerves. Other possible explana-

tions include reactivation of latent herpes simplex type 1 infections of the geniculate ganglia of facial nerves [101, 102].

In those who received the Pfizer-BioNTech vaccine, an increased risk of haemorrhagic stroke was observed (60 excess cases per 10 million) within 28 days post-vaccination. No increased risk for haemorrhagic stroke was seen in those who received AstraZeneca-University of Oxford vaccine. Similarly, the pathogenesis underlying the disparity in risk between both vaccines remains unclear. Interestingly, SARS-CoV-2 vaccines have been associated with increased risk of immune thrombocytopenic purpura [103, 104], which could contribute to major bleeding events. Whilst Patone et al. [100] concludes that haemorrhagic strokes are not increased in AstraZeneca-University of Oxford vaccine, several reports have identified the link between AstraZeneca-University of Oxford vaccine and cerebral venous sinus thrombosis with thrombocytopenia, now termed vaccine-induced immune thrombotic thrombocytopenia (VITT) [105–107].

In the UK, 438 VITT cases have been reported following 24.9 million first-dose vaccinations of AstraZeneca-University of Oxford, with 44 cases seen following the second dose. The Medicines and Healthcare products Regulatory Agency (MHRA) had reported that 220 out of 438 reports occurred in females, whilst 214 occurred in males aged from 18 to 93 years. Currently, the case fatality rate is 18% ($n = 79$ deaths), of which six occurred following the second dose. As of 9th February 2022, the MHRA has reported 35 VITT cases following mRNA vaccines, where a case fatality rate of 13% ($n = 4$) was reported following the first dose [108]. Association of VITT development and age seems to be dependent on the dose number. For instance, a higher incidence rate was observed in the younger age groups (18–49 years; 21.4 per million doses) than those aged over 50 years of age (11.1 per million doses), whereas the reverse is observed in those receiving the second dose, as older groups are associated with higher incident rates (2.1 per million doses). It should be stated that these incidence rates following the second dose should not be directly compared to the first dose, as the time for follow-up and case identification is more limited and differs across the age groups [108].

The exact VITT pathogenesis remains unclear; however, VITT is an autoimmune condition, characterized by antibodies which activate platelets, evoking thrombosis within the arterial and venous circulation. Individuals with VITT exhibit high titre IgG antibodies against platelet factor 4 (PF4); a molecule stored within α-granules of platelets and is released during platelet activation [105–

107]. PF4 may have a role within the innate immune response, as it is shown to opsonize polyanionic surfaces of pathogens, which enables binding of anti-PF4 antibodies, produced by preformed B cells [109, 110]. However, in VITT, anti-PF4 antibodies confer a different role, as it is binding to the platelet FcγRIIA receptor, which induces downstream intracellular signalling causing platelet acivation and release of procoagulant platelet microparticles [111]. These procoagulant microparticles express tissue factor, which enhances the procoagulant state of the vasculature. The release of tissue factor could explain the propensity to cerebral venous sinuses thrombosis in VITT, as the tissue factor plays a pivotal role in thrombogenesis within the cerebral venous system [112].

The trigger for autoantibody formation by adenoviral vector vaccines remains ambiguous. Plausible hypothesis includes that the viral capsid of these vaccines could bind to PF4, thus creating a novel antigen, which could be taken up monocytes and trafficked into lymph nodes, consequently stimulating proliferation of anti-PF4 memory B cells [105]. Such immune response may be potentiated through the production of a proinflammatory milieu, evoked by vaccine components such as edetic acid. Other hypothesis includes the presence of spike splice variant transcripts [113], whereby production of alternative spike protein could cause endothelial damage rendering inflammation, platelet activation, and thrombosis. However, no variant transcripts have been currently detected following vaccination with adenoviral vector SARS-CoV-2 vaccines.

Use of Monoclonal Antibodies Therapeutics in COVID-19

While vaccines remain the best arsenal to prevent COVID-19, monoclonal antibodies (mAbs) that can bind and "neutralize" SARS-CoV-2 in infected patients represent a novel class of antiviral intervention [114, 115]. Neutralizing mAbs are recombinant proteins, which can be derived from B cells of either convalescent patients or humanized mice. Application of high-throughput screening of these B cells enables identification of antibodies comprising the required specificity and affinity to attach to SARS-CoV-2 and inhibit cellular entry. Consequently, this can abrogate substantial SARS-CoV-2-induced pathology. Such mAbs are defined as neutralizing and can be used as a type of passive immunotherapy to reduce SARS-CoV-2 virulence.

The use of mAbs in SARS-CoV-2 has been extensively reviewed by Taylor et al. [116]; thus, an overview, in relation to vaccines, will be provided here. In the UK, three

mAbs have been licenced for COVID-19. This includes Ronapreve (REGN-COV2), which is a combination therapy of casirivimab with imdevimab [117], along with sotrovimab [118], whereas the USA have authorized the emergency use of REGN-COV2, bamlanivimab as monotherapy, or combined with etesevimab [119–121]. REGN-COV2 comprises two IgG1 mAbs with unmodified Fc regions. These mAbs bind two distinct and non-overlapping regions on the RBD [119, 122]. These anti-RBD mAbs prevent a spike protein to bind to ACE2 on target cells, consequently suppressing viral entry and subsequent infection. The combinative approach was based on the rationale that it is unlikely that a spike mutation would render resistance to both antibodies simultaneously. This has been reinforced in in vitro settings, where the combinative therapy retained its neutralizing capacity to all known spike protein mutations [122]. It was further shown that binding of REGN-COV2 to RBD domain evokes both antibody-mediated cytotoxicity and cellular phagocytosis in virally infected cells [119]. In an ongoing phase III placebo-controlled trial (NCT04425629), REGN-COV2 was shown to reduce viral loads in seronegative patients who initially had elevated SARS-CoV-2 viraemia [119]. Furthermore, post hoc analysis demonstrated that individuals treated with REGN-COV2 had lower cases of hospitalization, following SARS-CoV-2 infection, when compared to placebo. Reassuringly, the absolute risk reduction for REGN-COV2 was greater in high-risk patients for severe COVID-19 than placebo. Collectively, these findings support REGN-COV2 as a prophylactic measure, and to reduce hospitalization rates by promoting resolution of symptoms pertaining to acute SARS-CoV-2 infection.

It remains a tenet that neutralizing mAbs are most optimal when used early, as early viral load data support the notion to provide mAbs following a positive test or a symptom onset. However, in real-life scenarios, this is not feasible and not cost-effective to utilize such approaches in a generalist manner. Instead, a better pathway includes the selection of patients who would reap the greatest benefit, such as those who are expected to have poor antiviral responses (elderly or immunosuppressed) and an alternative for the unvaccinated. Furthermore, SARS-CoV-2 variants of concerns can prove resistant to neutralizing mAbs. Such was evident in the recent emergence of B.1.1.529, the Omicron variant [123, 124], which has shown as the predominant variant in several nations. It was shown that REGN-COV-2, bamlanivimab and etesevimab, lost significant neutralizing activity against the Omicron variant [125]. Consequently, this forced the

Food and Drug Administration to retract the use of these mAbs against Omicron and limit use to variants which are susceptible to these therapeutics.

Vaccination Responses in the Immunocompromised

As evident in this review, SARS-CoV-2 vaccines have demonstrated efficacy and effectiveness in clinical trials and population-level studies. Nevertheless, individuals enrolled onto these studies were predominantly healthy without any known chronic conditions. Consequently, there is an urgent need to characterize vaccine immunogenicity in patients with primary and secondary immunodeficiencies. From prior vaccine studies, patients with impaired immune responses elicit defective humoral responses to influenza and pneumococcal vaccinations, especially evident in those patients receiving B-cell-depleting agents such as rituximab [126, 127]. Moreover, immune responses towards vaccinations may be dependent on the type of immunosuppressive regimens and vaccine type received. However, findings from such studies may not be translatable into the use of the novel vaccines, which have been deployed against COVID-19.

A preprint produced by Public Health England [128], now known as UK Health Security Agency (UKHSA), evaluated serological responses in clinical high-risk groups in a nested test-negative case control cohort study. Data involving RT-PCR swab results and corresponding antibody results were collected from an electronic health record from 718 English general practices, which represented 10% of the population. Spike serological responses in 1,539 first-dose vaccinated adults (28 days post-vaccination), with no prior SARS-CoV-2 infection, were recorded. Within the specific clinical risk groups, it was demonstrated that the immunocompromised group had the lowest levels of seropositivity (70%) compared to non-immunocompromised individuals (95%). Following second dose, a 68% reduction in antibody titres was observed along with the next lowest responders: chronic respiratory groups (65% reduction), whilst these findings may potentially demonstrate lower humoral responses in these groups; there are certain limitations. Firstly, this observational study utilized a test-negative control design, where SARS-CoV-2 cases were initially confirmed by the lateral flow within the community and RT-PCR was used as a confirmatory tool; this could induce a temporal bias. Furthermore, T-cell responses were not evaluated.

To gain a better understanding of vaccine responses in individuals with impaired immune responses, the UK government employed the OCTAVE trial [129]. The OCTAVE study is a multicentre, prospective cohort study, which examines the SARS-CoV-2 vaccine responses in patient groups which are classed as immunosuppressed, immune-mediated inflammatory, and chronic diseases. Around 40% of enrolled patients were end-stage renal disease requiring haemodialysis (ESRD-HD), whereas those receiving immunosuppressive therapies were represented by inflammatory-bowel disease (21.8%), solid cancer and haematological malignancies (5.36%), inflammatory rheumatic diseases (28.00%), and haemopoietic stem cell transplant recipients (HSCT) (6.17%). These disease cohorts were selected as they potentially modulate SARS-CoV-2 vaccine responses by their underlying pathophysiology or their therapeutic management with immune-modifying treatments, such as biologics, disease-modifying antirheumatic drugs, T cell-mediated immunosuppressants (tacrolimus), and glucocorticoids. As of 23 August 2021, 2,592 patients were enrolled into the study where humoral and cellular responses to SARS-CoV-2 vaccines were assessed. Most of the participants had received the Pfizer/BioNTech or AstraZeneca-University of Oxford vaccine. An interim report provided results of the first 600 participants where serological data, measured by SARS-CoV-2 anti-spike assays, were reported at baseline, pre-second vaccine dose, and 4 weeks post second dose. The study demonstrated that 89% of patients seroconverted within 4 weeks post second dose, whereas 11% of patients across all disease groups failed to seroconvert after two doses. Failure to seroconvert post-two vaccine doses was predominantly found in patient subgroups, such as ANCA-associated vasculitis (AAV) (72.4%) and ESRD-HD receiving immunosuppression (16.7%). It should be highlighted all AAV patients had received rituximab, a monoclonal antibody targeting CD20 on B cells, which could explain failure to seroconvert following SARS-CoV-2 vaccinations. Furthermore, in comparison to healthy participants, 40% of patients, across multiple disease cohorts, demonstrated lower spike-reactivity following two vaccine doses. The significance of such level with protective immunity is yet to be elucidated. Strikingly, spike-specific T-cell responses were similar between disease groups and healthy controls. Such observation was demonstrated by a cohort study conducted by Mohanraj et al. [130], where SARS-CoV-2-specific T-cell responses were similar, in renal transplant and haematological malignant patients compared to healthy controls.

Whilst the OCTAVE study does produce reassuring evidence that patients with impaired immune responses

do respond to the vaccine; it is an ongoing study. Therefore, the vaccine effectiveness, not vaccine efficacy, is not yet available, as clinical infection data in these participants have not been assessed yet. These will be linked over time, as National Health Service records will be amalgamated with this study. Thus, the functionality of these responses cannot yet be derived. Importantly, there is no clinically validated cut-off for serological responses, which correlates with clinical protection. Interestingly, within the healthy cohorts, the lowest titres all exceeded 380 U/mL. However, 87% of AAV, 42% of ESRD-HD with immunosuppression, 33% with haematological malignancies, and 17% of haemopoietic stem cell transplant recipients were all below this level following two doses. Quantitatively, this may demonstrate that disease groups have lower humoral responses than healthy controls. This may be an important factor as antibodies wane over time and cross-protectivity is required against new-emerging variants such as Omicron.

Other limitations in this study exist, as baseline pre-vaccination data were not available due to the rapid deployment of vaccination programme. Moreover, healthy controls were healthcare workers with a female predominance, and age groups were poorly matched. The study also realizes that heterogeneity in terms of vaccine responses but also therapeutic management and inter-lapping comorbidities may all skew vaccine responses. Future studies must control for these factors if insights into protective immunity are going to be derived from this clinical group.

Following this, UK Research and Innovation and the UK government vaccine task force have co-founded a new study, OCTAVE Duo, which investigates the efficacy of a third dose ("booster dose") in those with absent or low antibody responses. Interim results have yet to be released. Both patients and clinicians would be eagerly awaiting to see what effect, if any, the top-up dose has on these poor-vaccine responders. The topic of booster doses has been contentious between governments and WHO, the latter criticizing booster vaccination in healthy individuals, whilst developing countries still await their first dose. Whilst SARS-CoV-2 is a novel virus, the old-age disparity of vaccine inequality continues.

Conclusions

Over the last 18 months, the world has been completely rewired and had to adjust with the new normal. Daily infection numbers and deaths from SARS-CoV-2 are a bleak normality, but there is one glimmer of positivity in this devasting pandemic: the triumph of science and scientists working as one united front. Whilst there are many unturned stones regarding the COVID-19 vaccine effort, which requires urgent addressing for current and future pandemics, the development and rollout of novel vaccinology are remarkable. Crucial questions still linger: What constitutes protective immunity? How do we know the clinically vulnerable are protected from SARS-CoV-2? Will SARS-CoV-2 immunizations be the new annual jab to keep up the race with emerging variants? Unfortunately, answers to these remain elusive. However, we believe that large global trials involving enrolment of immunosuppressed patients are required. Cellular and humoral responses along with vaccine effectiveness must be followed over an extended period. Efficacy is largely known, and it is now time to explore vaccine effectiveness in both healthy and immunosuppressed individuals, to finally answer the question, am I fully protected against COVID-19? When the global response is yes, we might then regain the life we once knew before SARS-CoV-2 loomed on mankind.

Conflict of Interest Statementt

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Author Contributions

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